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Xanthine Dehydrogenase (XDH) Assay Kit (Cat #: E-154)

COMPONENTS: XDH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
10x XDH Reaction- 0.5 ml, store at -20ºC
10x XDH Control- 0.5 ml, store at -20ºC
10x Tissue Lysis Solution- 25 ml, store at 4ºC **(dilute 10x with ice-cold dH_2O)**

PRODUCT DESCRIPTION: The XDH enzyme activity assay uses hypoxanthine as substrate and is based on the reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for measurement of XDH activity present in tissue/cell extracts. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Tissue Lysis Solution by diluting 10x Tissue Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50–100 µl ice-cold 1x Tissue Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Tissue Lysis Solution (~5 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. The suggested sample protein concentration is 0.5 − 3 mg/ml. *Normalization of sample protein concentration is recommended.*

Reagent thawing:

Thaw XDH Assay Solution and keep solution on ice and shielded from light. *Do not over thaw.* Freeze solution immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of 10x XDH Control and 10 parts of XDH Assay Solution, e.g. 50 µl 10x XDH Control and 500 µl XDH Assay Solution.

Reaction solution is prepared by mixing 1 part of 10x XDH Reaction and 10 parts of XDH Assay Solution, e.g. 50 µl 10x XDH Reaction and 500 µl XDH Assay Solution.

Enzyme assay:

1. Calculate the amount of control and reaction solutions required for each assay and prepare the solutions as described above.
2. Use a plain (uncoated) 96-well plate for the assay. Add 20 µl of each sample to duplicate wells of the plate (for control set and reaction set).
3. Add 50 µl control solution to one set of sample wells and 50 µl reaction solution to the other set of sample wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C incubator for 60 min or 120 min. *Do not use CO_2 incubator.*
4. Measure $O.D._{492\ nm}$ using a plate reader. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
5. If incubation for 60 min, XDH enzyme activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl/(60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.1**. If incubation for 120 min, XDH activity = **ΔO.D. x 4.05**. Enzyme activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity (see cat#E-101).

Additional information:

Both the assay and substrate solutions contain dimethyl sulfoxide (DMSO). The assay solution also contains iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.